REMARKS

This responds to the Office Action mailed on April 3, 2009.

Claims 1-7 were rejected under 35 U.S.C. § 103(a) as being obvious over Kreutzer et al. (U.S. Publication No. 2004/0001811), Elbashir et al. (Methods, 26:199 (2002)), Nilsen et al. (U.S. Patent No. 6,013,447), De Young et al. (Biochemistry, 33:12127 1994)), Hernandez (EMBO, 4:1827 (1985)), and Skuzeski et al. (J. Biol. Chem., 259: 8345 (1984)). This rejection is respectfully traversed.

Kreutzer et al. disclose a double stranded (ds) RNA with a RNA strand having a region that is less than 25 nucleotides in length and complementary to at least a portion of a RNA transcript of an anti-apoptotic gene such as Bcl-2. Kreutzer et al. also disclose that the individual strands of a dsRNA can be expressed from two separate vectors or from the same vector, or as an inverted repeat joined by a linker polynucleotide so as to form a stem and loop structure, and that the promoter can be a PolI promoter, PolII promoter, PolIII promoter or a prokaryotic promoter. However, the ssRNAs used to prepare dsRNAs for transfection in the Examples were prepared by conventional oligonucleotide chemical syntheses.

Although Kreutzer et al. mention a U1 snRNA promoter in paragraph 0089 as an example of a RNA polymerase II promoter, paragraph 0089 also discloses that an eukaryotic RNA polymerase I promoter, e.g., a rRNA promoter, a CMV promoter (a RNA polymerase II promoter), or preferably a RNA polymerase III promoter, e.g., an U6 snRNA or 7SK RNA promoter, or a prokaryotic promoter, may drive dsRNA expression.

The Examiner concedes that Kreutzer et al. do not teach siRNAs having UU 3′ overhangs or U1 snRNA termination sequences.

None of the dsRNAs in Example 1 of Kreutzer et al. form a molecule with an end having (nucleotides that can base pair are shown in bold immediately above or below the complementary base):

5'- **A/G**---------
3'-UU**U/C**---------

as recited in claim 1.

Rather, the dsRNAs in the Example in Kreutzer et al. have the following ends:

5′-**CAGG**—————**GA**CC-3'
3′-CG**GUCC**—————**CUGG**-5'

5′-**GCC**——————**GCC**-3'
3′-UA**CGG**——————**CGG**-5' and

5′-CA**AGG**——————**GC**A-3'
3′-UCU**GUCC**——————**CG**-5'.

Elbashir et al. disclose the use of small interfering RNAs (siRNAs; 21 to 23 nucleotides in length), which are the products of RNase III digestion of dsRNAs formed with mRNA, to silence genes in mammalian cells. It is disclosed that the predominant siRNAs formed in cells are 21 and 22 nucleotide RNAs with symmetric 2 nucleotide 3′ overhangs (see Figure 4B which shows "TT" overhangs). Elbashir et al. disclose that 21 nucleotide RNAs useful to form siRNAs are prepared via conventional oligonucleotide chemical syntheses and annealed prior to transfection.

The Examiner asserts that Elbashir et al. teach siRNA with 3′ UU on page 202 (page 4 of the Office Action).

Applicant respectfully disagrees. Page 202 in Elbashir et al. discloses that, independent of the selection scheme in Figure 2, the sense siRNA should be synthesized as N19TT and the antisense siRNA as N′19TT, where T indicates 2′deoxythymidine. It is disclosed on page 201 of Elbashir et al. that the use of 2′deoxythymidine overhangs reduces the cost of siRNA synthesis, may enhance nuclease resistance, and ensures that the sequence-specific endonuclease complex is formed with an approximately equal ratio of sense to antisense RNA-cleaving complexes.

Nilsen et al. relate to vectors and methods to identify affector RNA molecules that inhibit expression of target RNA molecules (abstract).

The Examiner points to column 13 of Nilsen et al. as teaching a vector with U1 snRNA promoter and termination sequence.

Column 13, lines 42-62, in Nilsen et al. discloses that preferred promoters for expressing the targeting gene in eukaryotic cells are either RNA polymerase III promoters or RNA polymerase II promoters characteristic of snRNA genes. It is also disclosed that it is preferred that a complete transcription unit be used including a promoter and termination sequence. However, a U1 termination sequence is <u>not</u> specifically mentioned in column 13 of Nilsen et al.

De Young et al. disclose the use of vectors with a U1 snRNA promoter and U1 snRNA terminator sequence, and a T7 promoter and T7 terminator sequence, to express ribozymes.

Hernandez discloses a vector to detect the processing of U1 nuclear RNA, which is transcribed by PolII and is involved in mRNA splicing. The vector includes an internally deleted U1 gene expressed from the SV40 promoter/enhancer. The vector was introduced to cells and the resulting RNA analyzed. The results showed that the first U1 RNA precursor has a few extra nucleotides at the 3′ end which are shortened to form mature U1 RNA, and that a 13 nucleotide sequence 3′ of the coding region is required to direct the first step in the formation of the 3' end of U1 snRNA.

Skuzeski et al. disclose the identification of two regions at the promoter essential for transcription of human U1 RNA and that there is a *Bgl*II site immediately 5′ to the U1 coding region (Figure 5).

The Examiner asserts that it would have been obvious to one of ordinary skill in the art to make an expression vector capable of expressing a siRNA comprising 3' UU overhangs as taught by Elbashir et al. and that one of ordinary skill in the art would have looked to Elbashir et al. while making siRNA as taught by Kreutzer et al. in order to design the optimal siRNA which includes 3' UU overhangs (page 5 of the Office Action).

However, Elbashir et al. point to the advantages of using 3' TT overhangs and, as discussed above, the dsRNAs in Example 1 in Kreutzer et al. do <u>not</u> have 3' UU overhangs.

The Examiner also asserts that the limitation requiring the expression vector to have a position +1 A or G would be a matter of routine design choice based on the sequence being targeted and therefore would be obvious to one of ordinary skill in the art.

If, in fact, the nucleotide base at position +1 is based on the sequence being targeted, it could be <u>any</u> of A, G, C, or T, <u>not</u> just A or G. Moreover, Applicant's vector includes a sequence corresponding to the sense region of the mRNA to be silenced <u>which sequence is 3' to</u>

the position +1 A or G. The specification discloses that the G or A at position +1 in that vector is for transcription initiation.

None of the cited documents employ a U1 promoter to produce siRNA or miRNA from a vector. Instead, each of the documents relating to siRNA employs conventional oligonucleotide chemical syntheses to prepare individual ssRNAs and the U1 based vectors in Hernandez and Skuzeski et al. were employed to detect *cis*-acting elements in the U1 gene.

Moreover, none of the cited documents individually or in combination with each other disclose or suggest an expression vector that provides for a double stranded molecule having the following end:

5'-**A/G**---------
3'-UU**U/C**--------- .

Applicant's specification discloses that the U1-based vectors of the invention have few sequence requirements at the 5' and 3' termini of the transcripts; they accept U sequences in the transcribed region (see above), unlike PolIII containing vectors; primary transcripts derived therefrom are efficiently exported to the cytoplasm and converted to the mature form; and specific sequences can be added at the 5' and 3' termini (see, for instance, claims 3 and 4), that allow the selection of only one of the two siRNA strands to be incorporated into the interference complex. This eliminates the accumulation of the sense strand that could mediate undesired targeting. In addition, as shown in Figure 3B, a vector of the invention provides transcripts that have specificity and provide for significant interference.

Accordingly, withdrawal of the § 103 rejection is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance, and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's representative at (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or deficiencies, or credit any overpayments to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being filed using the USPTO's electronic filing system EFS-Web, and is addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 2nd day of July, 2009.

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